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Identification of Morphological Characters of *Aquilaria microcarpa* in the Interaction with *Fusarium solani*

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Abstract

The principal mechanism acted in the formation of aloes on gaharu trees has not been well understood. The formation was thought to be one part of plant defense systems. The response of plants during interactions with pathogens is to react by synthesizing variety of toxic molecules both protein and non-protein molecules serving as protection against pathogens. Changes occurring in plants as a result of damage or stress were a response of induction causes. This study aimed to determine the effect of induction of the *Fusarium* spp inoculant (after 3 years) on the phenotypes of *Aquilaria microcarpa*. Based on observations and measurements of the general characters, results indicated that the inoculated *Aquilaria microcarpa* plants in Carita Banten had significant mean values higher than that of the un-noculated plants. In the inoculated plants, morphological character of total height was highly correlated with stems robustness, height of branch-free, diameter and volume of branch free. Meanwhile, in the observation and measurement of leaf, the width of leaf was highly correlated with length of leaf and length of petiol. Please do not alter them.

Keywords: *Aquilaria microcarpa*, *Fusarium solani*, gaharu, and morphological characters.

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1. Introduction

Aquilaria microcarpa Bail plant has erect stems and may attain a height of 40 m, diameter 2.5 m with compound leaves arranged special shaped oval, pointed, spiky, green and shiny green. Form of compound interest, situated at the tip of a twig (terminal) or in the axil of a leaf [1]. Generally, these plants grow well in fertile soil conditions and acid soil conditions even in the area of peat forest, swamp forest, lowland forest, forest or mountain with sandy soil texture and are also capable of growing in the cracks of rocks [2]. Plant of *A. microcarpa* Bail is one of the crop of aloes, but not all of this plant type would yield aloes. Formation of agarwood in plants is thought to be the mechanism of plant defense against biotic and abiotic factors. Abiotic factors such as the provision of chemicals treatment and mechanical on the stem lead to scarring of the plants react to the treatment which later formed the agarwood [3], however this could not lead to the spread to other parts of the tree that is not exposed to the direct effects. This is in contrast to biotic factors such as mushrooms or other miniscule body, agarwood formation mechanism may spread to other parts of the tree, because the cause of this mechanism is a living thing which does the necessary activities for life. With the onset of the spread formation of agarwood into other networks on a tree trunk, then the quality and quantity of the generated agarwood product will be more satisfying [4].

Various studies have been conducted on the plant producing agarwood from *Aquilaria* genus, especially in terms of cultivation and production engineering of agarwood [5]. The efforts were taken to meet the needs of increasing demand for agarwood in line with the rise in the selling price of agarwood. Attempt of cultivating plants producing agarwood in Indonesia has begun since 1994/1995 by a company exporter of agarwood, by planting *A. ardesiacus* covering an area of 10 hectares [4]. However, it was found several cases of unsuccessful entrepreneurial agarwood caused by failures in maintenance, failures in performing the inoculation and estimated time of logging as well as fitness of gaharu plant which is not appropriate, so that the results obtained are not satisfactory.

In general, harvesting of crops that allegedly having gaharu was done based on the following characteristics; I) yellow and leaf loss, II) small and thin tree canopies, III) break of a lot of tree branches, IV) bumps and grooves along the stem or branch of a tree, V) dried bark with brittle and break easily when pulled [7]. Prediction of plant having gaharu was done in areas of Dayak Kenyah and Punan in East Kalimantan by way of slicing and chopping, the wood of the agarwood-producing plants were exposed to infectious diseases in the central part of the stem [4]. But often times, these indicators were not appropriate in the suspected presence of agarwood because once those plants were cut down, there was no expected agarwood. Therefore, the research related to gaharu formation by identifying morphological characters of plants that interact with *F. solani* was required in order to produce agarwood.

2. Materials and Methods

The research was carried out in February 2012 to March 2012 in forested areas With special purpose (KHDTK) of Banten with Carita coordinates 06°8'-06°14' S and 105°50'-105°55' E. Plant *A. microcarpa* was introduced

from village Kampar district Kampar - Riau, planted in 1998. In 2009 several plants were inoculated with some *F. solani*.

Plant material used in this study was the whole plant *a. microcarpa* in Banten, Carita KHDTK as many as 110 trees consisting of 44 uninoculated trees and 66 inoculated trees. The instrument used was haga meters, meter tape, caliper, camera, tally sheets, and stationery.

Morphological observation was conducted in *A. Microcarpa* plants ranging from stem, branching, and leaves either on a plant which had been inoculated and uninoculated with *F. Solani*. Method used was descriptive non experimenter from about seventeen phenotypic characters(characters stems and leaves) on all trees of *A. Microcarpa*.

Determination of the plots examined description refers to several studies of forest plant variations performed by [6,7,8]. Measurement method can be seen in Fig. 1 and detailed measurements of the description presented in annex 1. Morphological measurement covered the measurement of stem total height (TTB), height of free branch (TBC), stem hardness, diameter (KkB), thick skin (TK), straightness of stem form (of the outbreak), stem quality (KB), free volume branch (VBC), the number of branches, the angle of the first branch of the shaper's header (ScP), the length of the leaves (PAD), the width of the leaves (LAD), and the length of the petiol (PP).

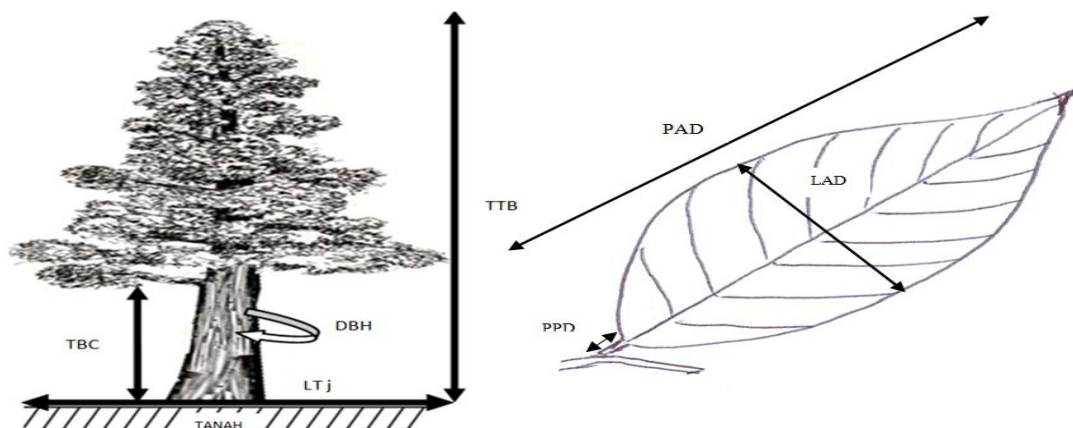


Figure 1. Technique to measure tree and leaves parameters

Differences in phenotypic appearance of the parent tree analyzed by testing the difference between the middle (compare the mean) on each character measured then conducted further trials of Tukey. The statistical parameters calculated include the middle value (average value), the standard deviation and the coefficient of variance.

Analysis of variance of phenotype in the population of *a. microcarpa* is done by looking at the correlation between the characters according to the formula of the correlation of Pearson. To outlining the structure of the variance over the linear combination of the variables (phenotypic characters) are measured, performed analysis factors. The pattern of clustering among the characters displayed in the form of a graph biplot intercultural factors using MINITAB program 15.0.

3. Results and Discussion

Visually morphology of plants a. *Microcarpa* good that has been inoculated three years and the was inoculated, on khdtk carita banten not showing absence of difference, as in the figure 2



Figure 2. Morphological features of inoculated (a) and uninoculated (b) *Aquilaria microcarpa*

Morphological observations of 17 plant a. *microcarpa* descriptors that have been inoculated or not inoculated, on samples of plants of a. *microcarpa* totalling 110 trees in KHDTK Carita Banten shows that there is a real difference except on high character and the first branch point forming the heading as shown in Table 1.

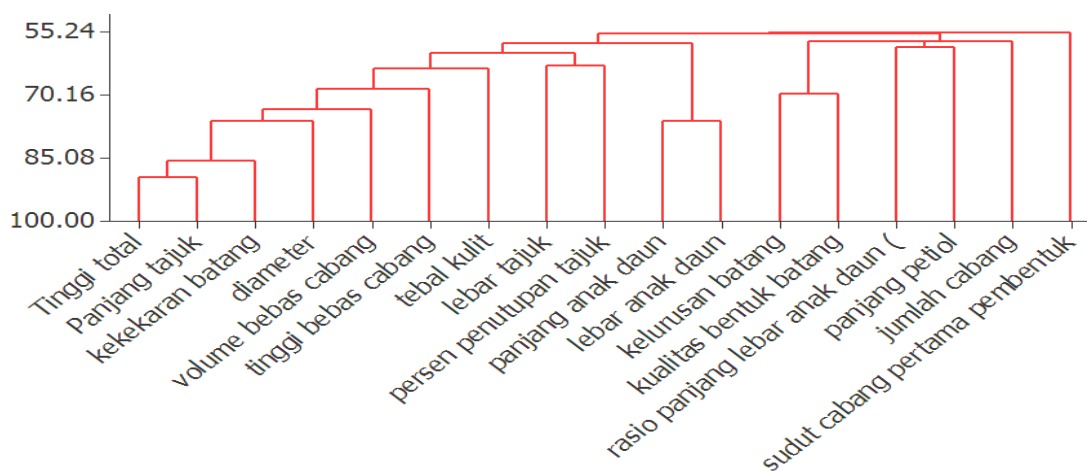
This research result indicated that in general the morphological characters of inoculated plants were not significantly different with the morphological characters of uninoculated plants. However, the results of observations on morphological character of total height obtained were higher than uninoculated plants. Moreover, morphological character of angle in the first branch of canopy form exhibited very significant differences, where the mean value indicated that the result measurements were lower at inoculated plants than that of uninoculated plants Both mean values of this character has links, where growth tall plant will be quickly if angles branches having angles smaller compared to plants having values of large angles.

Testing the level of similarity between the inoculated and uninoculated plants to determine the relationships of the morphological characters. The results showed that the degree of similarity of all morphological characters observed were 55,24% or diversity of 44,76% and these characters form the three groups. The first group consists of following characters: total length, hardness, stem diameter, volume, branch-free, thick skin, the canopy width and the canopy coverage. The second group consists of two characters namely the length and the width of leaves, while the third group was made up of six morphological characters namely straightness of stem, quality of stem form, ratio length and width of leaves, length of petiol, the number of branches and the angle first branch (Fig. 3). The measurement of morphological character having a high level of similarity (89.71%) is characters of total height and length of canopy.

Table 1: Mean and standard deviation of morphological characters in inoculated and uninoculated *Aquilaria microcarpa* at KHDKT Carita.

| Observed characters | Measurement unit | Uninoculated (n = 44) | Inoculated (n = 66) | P value |
|-------------------------------------|------------------|-----------------------|---------------------|---------|
| Total height | m | 8.273 ± 2.74 | 9.288 ± 2.41 | 0.043* |
| Height of free branches | m | 3.023 ± 1.135 | 3.177 ± 1.251 | 0.512 |
| Diameter | cm | 23.56±10.79 | 26.17 ± 9.4 | 0.182 |
| Stem hardness | index | 2.941 ± 1.168 | 3.225 ± 1.226 | 0.228 |
| Skin thickness | mm | 0.2154 ± 0.063 | 0.2267 ± 0.0559 | 0.329 |
| Straighness of stem | index | 8.886 ± 0.387 | 8.7576 ± 0.4661 | 0.132 |
| Quaility of stem form | index | 10 ± 0,092 | 9.9394 ± 0.4924 | 0.349 |
| Volume of free branches | cm ³ | 0.00186 ± 0.00118 | 0.00225± 0.00169 | 0.194 |
| Height of canopy | m | 5.166 ±2.335 | 5.989 ± 2.41 | 0.078 |
| Width of canopy | m | 3.847±1.697 | 3.811 ± 1.414 | 0.904 |
| Number of branches | number | 8±3.816 | 8.561 ± 3.595 | 0.436 |
| Angle of free branch canopy form | (⁰) | 65.34±19.78 | 55.53 ± 15.54 | 0.004** |
| Canopy cover | % | 42.61±20.07 | 43.03±15.59 | 0.903 |
| Length of leaves | cm | 5.943±0.851 | 5.939±0.82 | 0.981 |
| Height of leaves | cm | 2.443±0.701 | 2.6288±0.6811 | 0.169 |
| Ratio of length and width of leaves | ratio | 2.585±0.691 | 2.3722± 0.5491 | 0.075 |
| Length of petiole | mm | 3.773±0.803 | 3.7575±0.7245 | 0.918 |

Description: * significantly different; ** highly significantly different.

**Figure 3.** Similarity dendrogram of morphological characters in *Aquilaria microcarpa*

Based on the results of data analysis on morphological characters of plants *A. microcarpa*, then to determine a key character that is capable of being a determiner diversity of inoculated and uninoculated, can be seen by the use of factor analysis. The value of the coefficients of factor analysis of each morphological character in inoculated and uninoculated plants (Table 3).

Table 3: Coefficient values from factor analysis of uninoculated and inoculated *A. microcarpa*

| Characteristic measured | Analysis factor | |
|---------------------------|-----------------|------------|
| | Uninoculated | Inoculated |
| Eigenvalue | 3.838 | 2.831 |
| Diversity (%) | 0.226 | 0.167 |
| Total height | 0.897 | 0.794 |
| Height of free branches | 0.255 | 0.434 |
| Diameter | 0.815 | 0.798 |
| Stem hardness | 0.413 | 0.275 |
| Thickness | 0.272 | 0.291 |
| Stem straightness | -0.206 | -0.004 |
| Quality of stem form | 0.222 | 0.001 |
| Free branch volume | 0.718 | 0.739 |
| Length of canopy | 0.784 | 0.558 |
| Width of canopy | 0.112 | 0.404 |
| Number of branch | 0.067 | -0.052 |
| Angle of first branch | -0.116 | 0.117 |
| Canopy coverage | 0.255 | 0.073 |
| Length of leaf | 0.363 | 0.027 |
| Width of leaf | 0.498 | 0.250 |
| Ratio of length and width | -0.329 | -0.285 |
| Length of petiol | -0.216 | -0.025 |

Analysis factor was used to determine which variable that affects the level of high diversity for factor selected from the results of the analysis is just one factor. Based on loading factor, the value in the factor 1 greater than | 0,5 | denoted the corresponding variables affect the characters of the plant that are not inoculated with a high diversity values such as overall height, diameter, volume of free branch, and the length of canopy. The inoculated plants on factors that have a high diversity values i.e. total height, diameter, stem hardness, length of branch and volume of free branch. The second measurement results show the value of the diversity of the same factor analysis at both inoculated and uninoculated plants.

The pattern of clustering among the morphological characters evaluated can be displayed in the biplot graph between observed factors. The biplot showed that the uninoculated trees were characterized by morphological characters of total height, height of branches, the diameter and length of canopy, while main morphological

characters of inoculated plants were total height, total height of free branches, diameter and canopies. Other measurement of morphological characters cannot be represented exactly as a variable to differentiate between inoculated and uninoculated plants.

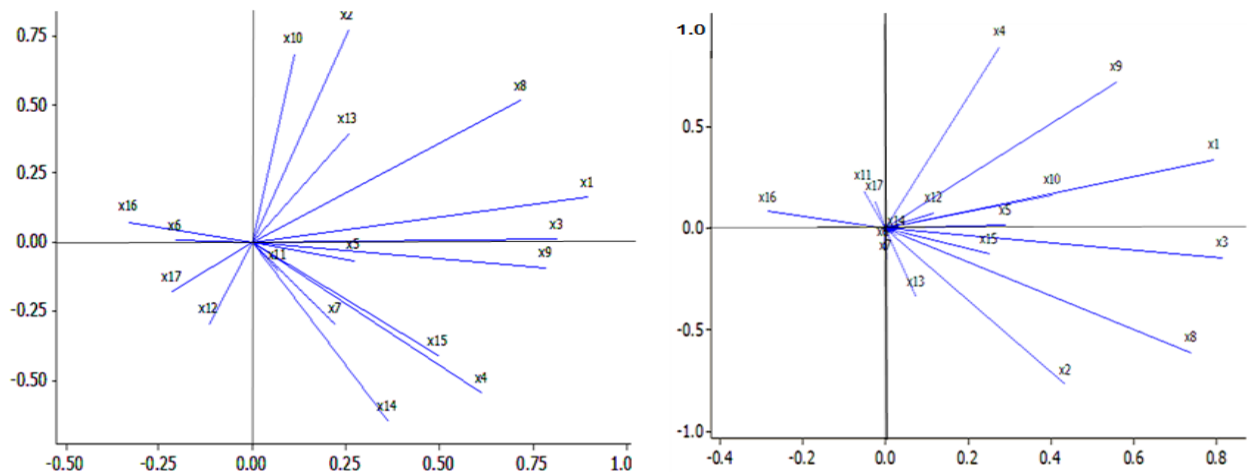


Figure 4. bi-plot morphological characters between inoculated (1) and uninoculated (2) *Aquilaria microcarpa* (X1=total height, X2= height free branch, X3= diameter, X4= branch rigidity, X5= bark thickness, X6= trunk straightness, X7= branch quality, X8= volume, X9= crown length, X10= crown width, X11= number of branch, X12= angle of first branch, X13= crown percentage coverage, X14= length of young leaf, X15= width of young leaf, X16= ratio of length and width of leaf, X17= petiole length)

Morphological characters were measured showed a high degree of similarity, between the plants that have been inoculated or not inoculated. It is thought to be because the body of the plant defense reactions occur, resulting in plants keep growing and growing. [9] States that the plant can be resistant to pathogens because the plant is in a group of plants that are immune to pathogens (non-host resistance = non-host resistance) or because the plant has to overcome resistance genes of pathogenic virulence (true = true resistance endurance) or because of some reason spared or plants tolerant of infection pathogens (real = apparent resistance endurance).

[10] stated that signs the establishment of aloes seeing from morphological factor up to six month observation was yellowing of the leaves and fall out; skin leafless, dried stems; twigs and branches start molting and easily broken; the stem; branches and twigs were white fibrous brownish black with a terrace wood brownish red or black if skin peeled; if its skin burned will issue scent of peculiar aloes.

[11] Mentioned that interaction with the plant pathogen may cause the onset of physiological changes on plants that have an impact on the occurrence of visual changes in the cells, tissues or organs of a plant. It can even result in changes to the morphology of plants [12,13]. The appearance of an organism morphology is the result of metabolic processes that occur in every cell of the organism.

Diversity of morphology on individual within a population depends heavily on diversity process and the result of metabolism occurring in each individual. The metabolic processes occurring in cell biochemical reactions were catalized by certain enzymes resulting in diversity morphology and the metabolism.

Variation susceptibility to a pathogen in plants also caused the differences in the number of survived genes [9]. Plant which is very susceptible to a isolates pathogenic; behold don't have the genes ketahanan effective to overcome isolates that diinokulasikan in plants, as a result, those plants perish if it pathogens diinokulasikan highly virulent. Plant A. Microcarpa who was inoculated with F. Solani, also have to die at was inoculated, though other plants in surrounding not subjected to the same thing.

The environment can affect the number and activity of the pathogen [14]. Plant pathogen virulence and susceptibility is not changed on the same plant for a few days to a few weeks, but the State of the environment can change suddenly in the depth varies. Therefore, the environment may also lead to the occurrence of change in disease progression becomes faster or slower. Of course the changes that occur in the environment factors capable of affecting plant host, pathogen or both. Changes in environmental factors it may be advantageous for the growth of pathogens and host for the plant unprofitable.

The plant selection technique will be inoculated against influential also alleged value of morphological characters of plant inoculated, where the plant will be inoculated generally are plants that are healthy phenotypes. This has resulted in the value of the measurement results of the morphological characters of inoculated plants higher than plants that have not been inoculated.

In 2008 at the site of the research, there was pest attack from *Heortia vitessoides* which resulted in an interruption in the process of formation of agarwood. As a result of the attacks, some producing agarwood tree experienced damaged leaves, trees being deciduous trees, even death. The stricken of *h. vitessoides* caterpillars caused many deaths plants occurred on the inoculated plant groups, since such attacks hinder plant growth and even cause the death of the plant.

The openness of the land caused by the demise of vulnerable plants that were not resistant to inoculation made caterpillar of *h. vitessoides* attacks and,gave opportunities for the surrounding plants to grow and flourish, even at some plants that were resistant to Fusarium, its morphological character measurement values were higher than those of other trees. But when viewed on the research results of this second group of plants inoculated or uninoculated characters total height measurement, diameter, length and branch-free volume headers were equally providing value a high diversity.

4. Conclusion

Measurement of morphological characters of plant inoculated or uninoculated factor analysis based on the retrieved value diversity measurements total height, diameter, length and branch-free volume headers were the same between the two groups. This indicates that morphological characters used, have not been able to describe the difference between an inoculated and uninoculated plant.

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